Full Paper

Ultrasound-Assisted Synthesis of Novel α-Aminophosphonates and Their Biological Activity

Syed Rasheed[1], Katla Venkataramana[1], Kuruva Chandrasekhar[1], Gemmalamadugu Fareeda[2], and Chamarthi Naga Raju[1]

[1] Department of Chemistry, Sri Venkateswara University, Tirupati, India
[2] Department of Plant Sciences, Hyderabad Central University, Hyderabad, India

The synthesis of a series of novel α-aminosubstituted phosphonates was accomplished by the reaction of various substituted aldehydes with an amine amlodipine (3-ethyl 5-methyl (±)2-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridene-3,5-dicarboxylate) followed by diethylphosphite/dibutylphosphite in ethanol using $SnCl_2.2H_2O$ as a Lewis acid catalyst, under conventional and ultrasonic irradiation. Their structures were established by analytical and spectral data. The title compounds showed good antibacterial, antifungal and antiviral activity depending on the nature of the bioactive groups at the α-carbon.

Keywords: Aminophosphonates / Antimicrobial activity / Antiviral activity / Pudovik reaction / Ultrasound irradiation

Received: July 18, 2011; Revised: October 1, 2011; Accepted: October 11, 2011

DOI 10.1002/ardp.201100256

Introduction

Phosphonic acids and their phosphonate derivatives are of immense interest in synthetic organic chemistry due to their biological activities [1]. They are employed in synthetic operations leading to carbon-carbon and carbon-phosphorus bond formation [2]. In recent years, considerable interest has been focused on the synthesis of α-aminophosphonates because they are considered to be structural analogues of α-amino acids. These compounds have several potential applications in medicine as anticancer agents [3], potential enzyme inhibitors [4], antiviral agents [5, 6], antibiotics and pharmacological agents [7], antitumour agents [8] and in agriculture as herbicides [9] and insecticides [10], as is well documented.

Having several potential applications, a variety of synthetic approaches [11] have been developed for the synthesis of α-aminophosphonates. Of these methods the nucleophilic addition of phosphonate containing labile hydrogen at the imine carbon catalysed by an acid or a base is one of the most convenient methods. Lewis acids [12, 13] are known to catalyze this reaction under mild conditions.

Amlodipine (3-ethyl 5-methyl (±)2-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridene-3,5-dicarboxylate), commercially available as Norvasc® is a long acting calcium channel blocker used as a hypertensive drug and the mechanism involved in it is a dihydropyridine calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. It selectively inhibits calcium ion influx across cell membranes, with a greater effect on vascular smooth muscle than on cardiac muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

The present paper describes the synthesis of α-aminosubstituted phosphonates by Pudovik reaction by conventional and under ultrasonic irradiation by one-pot reaction of amlodoipine with various substituted aromatic/heterocyclic aldehydes followed by diethyl/dibutylphosphite in dry ethanol with catalytic amount of $SnCl_2.2H_2O$. Nowadays ultrasonic irradiation is becoming a powerful tool for a synthetic organic chemist because of high yields and very short reaction times [14–16]. The effects of ultrasound on organic reactions are

Correspondence: Chamarthi Naga Raju, Department of Chemistry, Sri Venkateswara University, Tirupati–517502, India
E-mail: rajuchamarthi10@gmail.com

attributed to cavitations, a physical process that creates, enlarges and implodes gaseous and vaporous cavities in an irradiated liquid [17]. The cavitations induce very high local temperatures and pressures inside the bubbles (cavities), leading to a turbulent flow in the liquid and enhanced mass transfer [18]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several organophosphorus compounds for their potential biological activity.

Results and discussion

Chemistry

First we synthesized α-aminophosphonates by adopting the conventional method. This method displayed a few drawbacks such as longer reaction time and low yields. In order to optimize the reaction conditions, the synthesis of **4a** was carried out under a variety of conditions. Then it was determined that ultrasonic conditions should be used as the reaction without ultrasonic activation was much slower and, for example, the product was obtained in yield of 68% after 4 h of conventional heating using $SnCl_2.2H_2O$ as compared with a yield of 78% obtained after 20 min under ultrasonic irradiation (75°C).

For an obvious examination of the influence of ultrasound irradiation in this reaction, comparison of the reaction under two methods, conventional method (method A) and ultrasonic irradiation (method B) were performed. As illustrated in Table 1, method B is comparatively better than method A in both yield and especially in the reaction times.

The chemical structures of all the title compounds **(4a–l)** were characterized by IR, ^1H, ^{13}C, ^{31}P NMR and mass spectral studies and their data are presented in the experimental section. Characteristic IR stretching absorptions were observed in the regions 3250–3400 cm^{-1}, 1215–1230 cm^{-1} and 737–765 cm^{-1} for N–H, P=O, P–$C_{aliphatic}$ [19]. In ^1H NMR and ^{13}C NMR spectra chemical shifts for amine were observed in their expected regions [20]. The P–C–H protons resonated as a doublet of doublet due to their coupling with phosphorus and neighbouring N–H proton. The N–H proton signal appeared as a triplet due to its coupling with neighboring proton and phosphorus [21, 22]. ^{13}C NMR spectra were recorded for six compounds and their data are presented in the experimental section. In ^{31}P NMR, signals appeared as singlets in the region 21.12–24.12 [23]. ESI mass spectra were recorded for six compounds and they gave $M^{+\cdot}$ ions.

The present method describes the preparation of α-aminophosphonates **(4a–l)** with increased yield and with short reaction times compared to conventional methods. These transformations were carried out without any significant amounts of undesirable side products.

Biology

Antibacterial, antifungal and antiviral activities

All the synthesized compounds exhibited prominent antibacterial, antifungal and antiviral activities (Tables 2–4). Aldehyde containing two halogens (**4b, 4c, 4f, 4g, 4h, 4l**) exhibited excellent antibacterial, antifungal and antiviral activity compared to the remaining compounds. Remaining aldehydes exhibited promising activities. In spite of the large number of antibiotics and chemotherapeutics currently available for medical usage, the problem of increasing bacterial resistance has made it necessary to continue the search for new antimicrobial substances [24].

Conclusion

In conclusion, we have synthesized a novel series of α-amino-substituted phosphonates by reacting amlodopine and various substituted aldehydes with diethyl, dibutyl phosphites by Pudovik reaction using stannous chloride as a catalyst in the conventional method. In the other method, same phosphonates with high yield in short reaction times under ultrasonic irradiation were reported. We believe that sonochemical syntheses of α-aminophosphonates may be considered as valuable contribution in the field of phosphorus chemistry as compared to the existing process. The compounds (**4b, 4c, 4f, 4g, 4h, 4l)** exhibited excellent antibacterial, antifungal and antiviral activity due to the presence of two halogens on the aldehyde moiety of α-aminophosphonates.

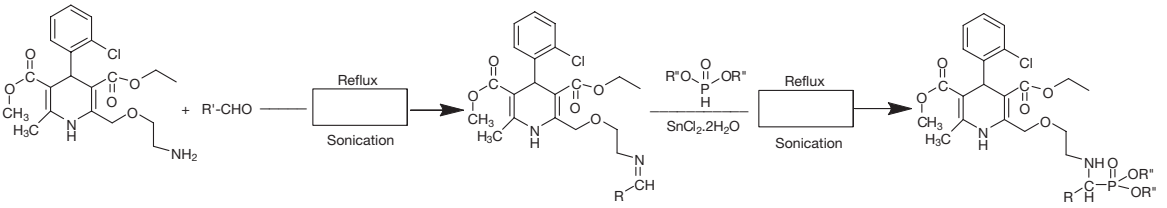
A definite SAR could not be established due to lack of structural diversity of the investigated compounds and further research needs to be performed to identify lead structures with better activity.

Experimental

Chemistry

All chemicals were procured from Sigma-Aldrich, Merck and were used as such. Solvents used for spectroscopic and physical studies were reagent grade and were further purified by the literature methods [25]. Melting points were deteremined in open capillary tubes by Guna digitial melting point apparatus expressed in (°C) and are uncorrected. Infrared spectra (υ_{max} in cm^{-1}) were recorded as KBr pellets on a Perkin-Elmer FT-IR 100 spectrophotometer. ^1H, ^{13}C and ^{31}P NMR spectra were recorded as solutions in DMSO-d_6 on a Varian 400 MHz spectrometer operating at 400 MHz for ^1H, 125 MHz for ^{13}C and 202 MHz for ^{31}P NMR. The ^1H and ^{13}C chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS) and ^{31}P chemical shifts to 85% H_3PO_4. ESI mass spectra were recorded on an API-3000 mass spectrometer. Elemental analysis was performed on a Thermo Finnigan instrument at the University of Hyderabad, Hyderabad. Sonication was performed using Bandelin Sonorex with a frequency of 35 kHz and a nominal power 200W ultrasonic bath for ultrasonic

Table 1.



Synthesis of α-aminosubstituted phosphonates (**4a–l**).

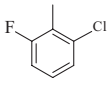
Compound	R	R^1	Method A	Method B
			Time (h)/Yield (%)	Time (min)/Yield (%)
4a		Et	4/68	20/78
4b		Et	4/78	21/87
4c		Et	4.5/72	22/82
4d		Et	4.5/74	22/88
4e		Et	5/69	20/78
4f		Et	6/70	24/82
4g		Bu	4.5/65	21/76
4h		Bu	4.5/80	22/88
4i		Bu	4.5/70	21/83
4j		Bu	5/66	19/84
4k		Bu	5.2/64	22/76
4l		Bu	6/68	23/84

Table 2. Antibacterial activity of title compounds.

Compound[a]	Zone of inhibition (mm)			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
4a	10.0 ± 0.38	12.0 ± 0.14	20.0 ± 0.25	9.0 ± 0.11
4b	13.0 ± 0.21	18.0 ± 0.70	28.5 ± 0.14	14.9 ± 0.24
4c	13.2 ± 0.70	17.4 ± 0.23	28.0 ± 0.51	14.2 ± 0.31
4d	11.0 ± 0.40	14.0 ± 0.54	26.0 ± 0.24	11.0 ± 0.24
4e	10.0 ± 0.71	14.2 ± 0.50	25.0 ± 0.32	11.0 ± 0.51
4f	14.0 ± 0.18	17.5 ± 0.64	20.7 ± 0.51	15.0 ± 0.48
4g	12.0 ± 0.52	15.8 ± 0.30	25.5 ± 0.42	11.1 ± 0.60
4h	14.0 ± 0.27	16.2 ± 0.12	29.0 ± 0.60	14.7 ± 0.30
4i	14.2 ± 0.80	17.0 ± 0.42	29.8 ± 0.20	14.3 ± 0.45
4j	09.0 ± 0.31	13.0 ± 0.20	19.0 ± 0.30	10.0 ± 0.52
4k	09.5 ± 0.54	13.2 ± 0.18	20.6 ± 0.41	10.0 ± 0.72
4l	18.0 ± 0.30	20.0 ± 0.41	26.4 ± 0.34	15.7 ± 0.30
Gatifloxacin [b]	12.3 ± 0.50	16.0 ± 0.30	27.3 ± 0.72	13.7 ± 0.42

Values are means ± SD of three replicates ($p < 0.05$)
[a] 100 μg/mL
[b] 100 μg/mL

irradiation with built in heating 30–80 (°C) thermostatically adjustable. The reaction vessel was placed inside the ultrasonic bath containing water.

General procedure

Method A: Conventional heating

A mixture of amlodopine **(1)** (0.5257 g, 0.005 mol) and pyridine-3-carboxaldheyde (**2**) (0.5250 g, 0.005 mol) and few grams of molecular sieves of 4 Å were taken in a round bottom flask, refluxed in anhydrous ethanol for 2 h to form the corresponding aldimine (Schiff's base). The formation of Schiff's base was indicated by TLC using hexane and ethyl acetate (1:3) as a mobile phase. Then catalytic amount of $SnCl_2.2H_20$ was added and diethyl phosphite (0.49 mL, 0.005 mol) was added to the aldimine and the reaction mixture was stirred at reflux temperature for 2 h. Progress of the reaction was monitored by running TLC (silica gel) using hexane and ethyl acetate (1:3) as a mobile phase at different time intervals. After the completion of reaction, the solvent was removed in a rota-evaporator at reduced pressure and the crude product obtained was purified by column chromatography on silica gel (60–120 mesh), using hexane and ethyl acetate (3:1) as an eluent to afford the pure 3-ethyl 5-methyl 4-(2-chlorophenyl)-2-[(2-(diethoxyphosphoryl)(3-pyridyl)-methyl)aminoethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate (**4a**), yield, 68%.

Table 3. Antifungal activity of the title compounds.

Compound[a]	Zone of inhibition (mm)		
	A. niger	*A. flavus*	*F. oxysporum*
4a	11.3 ± 0.21	14.2 ± 0.26	11.2 ± 0.52
4b	20.4 ± 0.40	21.2 ± 0.54	23.2 ± 0.28
4c	21.1 ± 0.21	17.8 ± 0.80	24.2 ± 0.39
4d	12.1 ± 0.20	09.3 ± 0.23	15.4 ± 0.26
4e	11.3 ± 0.32	14.5 ± 0.14	13.2 ± 0.42
4f	19.3 ± 0.43	17.8 ± 0.25	20.3 ± 0.73
4g	15.3 ± 0.56	13.2 ± 0.52	17.2 ± 0.53
4h	18.3 ± 0.25	17.9 ± 0.31	19.3 ± 0.60
4i	20.4 ± 0.34	20.4 ± 0.19	21.2 ± 0.40
4j	16.5 ± 0.56	16.0 ± 0.80	13.2 ± 0.51
4k	14.3 ± 0.70	12.0 ± 0.21	15.2 ± 0.24
4l	19.3 ± 0.54	18.5 ± 0.25	19.2 ± 0.53
Clotrimazole[b]	17.3 ± 0.60	16.7 ± 0.43	18.2 ± 0.26

Values are means ± SD of three replicates ($p < 0.05$)
[a] 500 μg/mL
[b] 500 μg/mL

Method B: Ultrasonication

In the first step of the reaction, amlodopine (amine), aldehyde and molecular sieves were taken in a round bottom flask, sonicated for 10 min in anhydrous ethanol at reflux condition to form aldimine. Then $SnCl_2.2H_20$ and phosphite were added to the reaction mixture and again sonicated for 10 min for completion of the reaction as it was confirmed by running TLC. The

Table 4. Antiviral activity of the title compounds.

Compound	Inhibition rate (%)
4a	40.8
4b	56.8
4c	55.4
4d	42.0
4e	38.0
4f	55.6
4g	41.8
4h	54.6
4i	55.0
4j	41.0
4k	39.0
4l	54.9
Ningnanmycin	53.8

reaction mixture was worked up as described above and the yield obtained was 78%.

3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-{[(diethoxyphosphoryl)(3-pyridyl)methyl)amino}-ethoxy)methyl)]-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate ***4a***

Dark red solid. Mol.Wt: 636. mp 210–212°C. IR (KBr) (ν_{max} cm^{-1}): 741 (P–C), 1230 (P=O), 3210 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.16 (t, 6H, $2CH_3$, J = 7.1 Hz, H-2″), 1.22 (t, 3H, CH_3, J = 6.5 Hz, H-12), 2.10 (s, 3H, CH_3, H-15), 3.18 (dd, 2H, CH_2, J = 4.1, 5.1Hz, H-9), 3.30 (s, 3H, OCH_3, H-14), 3.60 (t, 2H, CH_2, J = 6.8 Hz, H-8), 4.04–4.20 (m, 8H, $3CH_2$, H-11, H-7, H-1″), 4.40 (s, 1H, CH, H-4), 5.30 (dd, 1H, P–C–H, J = 2.0, 8.5 Hz, H-22), 5.90 (t, 1H, J = 7.0 Hz, NH), 7.00–7.50 (m, 8H, Ar-H), 8.20 (s, 1H, NH, H-1); ^{13}C-NMR (DMSO-d_6) δ (ppm): 15.2 (C-12), 16.6 (C-2″), 18.7 (C-15), 37.2 (C-4), 39.0 (C-9), 50.2 (d, P–C–H, C-22), 54.0 (C-14), 60.2 (C-11), 63.1 (C-1″), 67.8 (C-7), 68.0 (C-8), 103.1 (C-5), 103.7 (C-3), 123.2 (C-2′ & 5′), 126.1 (C-21), 126.8 (C-20), 127.4 (C-19), 128.0 (C-18), 131.0 (C-17), 142.7 (C-6), 144.3 (C-2), 145.5 (C-1′), 144.6 (C-16), 146.8 (C-3′ & 4′), 166.4 (C-10), 166.5 (C-13); ^{31}P-NMR (DMSO-d_6) δ (ppm): 22.14; ESI mass m/z (%) 636 (100) [$M^{+\cdot}$], 638 (32) [$M^{+\cdot}$+2], 599 (10), 589 (10). Anal. cald. for $C_{30}H_{39}ClN_3O_8P$: C: 56.65, H: 6.18, N: 6.61. Found C: 56.50, H: 6.14, N: 6.45.

3-Ethyl-5-methyl 2-[(2-{[(2-chloro-6-fluorophenyl)(diethoxyphosphoryl)methyl]amino}-ethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5 pyridene dicarboxylate ***4b***

Brown solid. Mol.Wt: 687. mp 222–223°C. IR (KBr) (ν_{max} cm^{-1}): 749 (P–C), 1225 (P=O), 3365 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.15 (t, 6H, $2CH_3$, J = 6.8 Hz, H-2″), 1.29 (t, 3H, CH_3, H-12), 2.10 (s, 3H, CH_3, H-15), 3.00 (dd, 2H, CH_2, J = 4.0, 5.2 Hz, H-9), 3.30 (s, 3H, OCH_3, H-14), 3.74 (t, 2H, CH_2, J = 7.0 Hz, H-8), 4.03–4.21 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.80 (s, 1H, CH, H-4), 5.28 (dd, 1H, P–C–H, J = 2.2, 9.0 Hz, H-22), 6.70 (t, 1H, J = 7.0 Hz, NH), 7.00–8.40 (m, 7H, Ar-H), 8.50 (s, NH, H-1); ^{13}C-NMR (DMSO-d_6) δ(ppm): 15.4 (C-12), 18.2 (C-2″), 18.8 (C-15), 37.4 (C-4), 39.1 (C-9), 48.3 (d, P–C–H, C-22), 54.0 (C-14), 60.2 (C-11), 63.1 (C-1″), 67.6 (C-7), 68.2 (C-8), 103.2 (C-5), 103.5 (C-3), 114.2 (C-3′), 123.4 (C-1′), 126.2 (C-5′), 126.4 (C-20), 127.4 (C-19), 128.2 (C-18), 130.1 (C-21), 131.1 (C-17), 131.8 (C-4′), 133.8 (C-6′), 142.3 (C-16), 142.7 (C-6), 144.4 (C-2), 158.2 (C-2′), 166.8 (C-13), 167.0 (C-10); ^{31}P-NMR (DMSO-d_6) δ (ppm): 21.12. Anal. cald. for $C_{31}H_{38}Cl_2FN_2O_8P$: C: 54.16, H: 5.57, N: 4.07. Found C: 54.09, H: 5.49, N: 3.99.

3-Ethyl-5-methyl 2-[(2-[(3-bromo-4-fluorophenyl)(diethoxyphosphoryl)methyl]-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate ***4c***

Light yellow solid. Mol.Wt: 732. mp 198–200°C; IR (KBr) (ν_{max} cm^{-1}): 737 (P–C), 1215 (P=O), 3365 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.15 (t, 6H, $2CH_3$, J = 7.3 Hz, H-2″), 1.28 (t, 3H, CH_3, H-12), 2.12 (s, 3H, CH_3, H-15), 2.90 (dd, 2H, CH_2, J = 4.0, 5.2 Hz, H-9), 3.50 (s, 3H, OCH_3, H-14), 3.64 (t, 2H, CH_2, J = 7.2 Hz, H-8), 4.08–4.24 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.90 (s, 1H, CH, H-4), 5.34 (dd, 1H, P–C–H, J = 2.3, 8.7 Hz, H-22), 6.48 (t, 1H, J = 7.4 Hz, NH), 7.41–8.20 (m, 7H, Ar–H), 8.30 (s, NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm): 22.00. Anal. cald. for $C_{31}H_{38}BrClFN_2O_8P$: C: 50.87, H: 5.23, N: 3.83. Found C: 50.78, H: 5.18, N: 3.72.

3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-[(diethoxyphosphoryl)(4-hydroxy-3-nitrophenyl)-methyl]amino}ethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate ***4d***

Orange solid. Mol.Wt: 696. mp 201–203°C. IR (KBr) (ν_{max} cm^{-1}): 741 (P–C), 1230 (P=O), 3200 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.13 (t, 6H, $2CH_3$, J = 7.3 Hz, H-2″), 1.30 (t, 3H, CH_3, H-12), 2.20 (s, 3H, CH_3, H-15), 2.90 (dd, 2H, CH_2, J = 4.0, 5.2 Hz, H-9), 3.40 (s, 3H, OCH_3, H-14), 3.71 (t, 2H, CH_2, J = 7.0 Hz, H-8), 4.06–4.24 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.60 (s, 1H, CH, H-4), 5.27 (dd, 1H, P–C–H, J = 3.0, 9.0 Hz, H-22), 6.90 (t, 1H, J = 7.4 Hz, NH), 7.20–8.30 (m, 7H, Ar–H), 8.1 (s, 1H, Ar–OH), 8.40 (s, NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm): 22.30; ESI mass m/z (%) 696 (100) [$M^{+\cdot}$], 698 (30) [$M^{+\cdot}$+2] , 650 (14), 721 (14), 681 (10). Anal. cald. for $C_{31}H_{39}ClN_3O_{11}P$: C: 53.49, H: 5.65, N: 6.04. Found C: 53.38, H: 5.64, N: 6.03.

3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-{(diethoxyphosphoryl)(2-thienyl)methyl]amino}-ethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate ***4e***

Black solid. Mol.Wt: 641. mp 188–190°C. IR (KBr) (ν_{max} cm^{-1}): 765 (P–C), 1227 (P=O), 3380 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.19 (t, 6H, $2CH_3$, J = 6.7 Hz, H-2″), 1.29 (t, 3H, CH_3, H-12), 2.40 (s, 3H, CH_3, H-15), 3.12 (dd, 2H, CH_2, J = 4.0, 5.2 Hz, H-9), 3.45 (s, 3H, OCH_3, H-14), 3.80 (t, 2H, CH_2, J = 7.5 Hz, H-8), 4.10–4.26 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 5.00 (s, 1H, CH, H-4), 5.31 (dd, 1H, P–C–H, J = 2.0, 8.4 Hz, H-22), 5.80 (t, 1H, J = 7.6 Hz, NH), 7.20–8.00 (m, 7H, Ar-H), 8.20 (s, NH, H-1); ^{13}C-NMR (DMSO-d_6) δ (ppm): 15.6 (C-12), 18.2 (C-2″), 18.9 (C-15), 37.1 (C-4), 39.0 (C-9), 50.3 (d, P–C–H, C-22), 54.0 (C-14), 60.1 (C-11), 61.5 (C-1″), 67.8 (C-7), 68.1 (C-8), 103.2 (C-5), 103.7 (C-3), 125.9 (C-21), 123.2 (C-2′), 126.0 (C-3′), 126.2 (C-20), 126.5 (C-4′), 127.2 (C-19), 128.0 (C-18), 131.0 (C-17), 137.4 (C-1′), 142.3 (C-16), 142.7 (C-6), 144.3 (C-2), 166.4 (C-10), 166.5 (C-13); ^{31}P-NMR (DMSO-d_6) δ (ppm): 23.44; ESI mass m/z (%) 641 (100) [$M^{+\cdot}$], 643 (30) [$M^{+\cdot}$+3], 419 (10), 369 (10).

3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-{[(2,4-dichlorophenyl)-(diethoxyphosphoryl)methyl]-amino}ethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate ***4f***

Green solid. Mol.Wt: 703. mp 211–213°C. IR (KBr) (ν_{max} cm^{-1}): 750 (P–C), 1224 (P=O), 3350 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.18 (t, 6H, $2CH_3$, J = 7.0 Hz, H-2″), 1.30 (t, 3H, CH_3 , H-12), 2.21 (s, 3H, CH_3, H-15), 3.00 (dd, 2H, CH_2, J = 3.9, 4.4 Hz, H-9), 3.14 (s, 3H, OCH_3, H-14), 3.83 (t, 2H, CH_2, J = 7.5 Hz, H-8), 4.20–4.29 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 5.10 (s, 1H, CH, H-4), 5.29 (dd, 1H, P–C–H, J = 1.9, 8.2 Hz, H-22), 6.20 (t, 1H, J = 7.3 Hz, NH), 7.20–8.00 (m, 7H, Ar–H), 8.20 (s, NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm): 22.33; ESI mass m/z (%) 703.6 (100) [$M^{+\cdot}$], 705.6 (97) [$M^{+\cdot}$+2], 707 (30), 709 (5), 698 (10), 684.2 (10), 665 (10).

3- Ethyl-5-methyl 4-(2-chlorophenyl)-2-[2-{[(dibutoxyphosphoryl)(3-pyridyl)methyl]amino}-ethoxy)methyl]-6-methyl-1,4-dihydro-3,5 pyridene dicarboxylate ***4g***

Dark reddish solid. Mol.Wt: 692. mp 200–202°C. IR (KBr) (ν_{max} cm^{-1}): 745 (P–C), 1235 (P=O), 3345 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 0.85–1.80 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12),

2.10 (s, 3H, CH_3, H-15), 3.16 (dd, 2H, CH_2, J = 4.0, 3.0 Hz, H-9), 3.30 (s, 3H, OCH_3, H-14), 3.62 (t, 2H, CH_2, J = 7.3 Hz, H-8), 4.04–4.20 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.40 (s, 1H, CH, H-4), 5.28 (dd, 1H, P–C–H, J = 1.9, 8.2 Hz, H-22), 6.80 (t, 1H, J = 7.5 Hz, NH), 7.00–7.50 (m, 8H, Ar–H), 8.30 (brs, 1NH, H-1); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.6 (C-4″), 15.4 (C-12), 18.6 (C-3″), 18.9 (C-15), 30.2 (C-2″), 37.4 (C-4), 39.1 (C-9), 50.2 (d, P–C–H, H-22), 54.2 (C-14), 60.4 (C-11), 61.0 (C-1″), 67.8 (C-7), 68.2 (C-8), 103.2 (C-5), 103.5 (C-3), 123.2 (C-2′ & 5′), 126.1 (C-21), 127.2 (C-19), 126.4 (C-20), 128.0 (C-18), 131.0 (C-17), 142.3 (C-16), 142.6 (C-6), 144.2 (C-2), 145.5 (C-1′), 146.8 (C-3′ & 4′), 166.2 (C-10), 166.5 (C-13); ^{31}P-NMR (DMSO-d_6) δ (ppm): 23.50; ESI mass *m/z* (%) 692 (100) [$M^{+\cdot}$], 694 (32) [$M^{+\cdot}$+2]. Anal. cald. for $C_{34}H_{47}ClN_3O_8P$: C: 59.00, H: 6.84, N: 6.07. Found C: 58.94, H: 6.72, N: 5.96.

*3-Ethyl-5-methyl 2-[(2-{[(2-chloro-6-fluorophenyl)-(dibutoxyphosphoryl)methyl]amino}ethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate **4h***

Brown solid. Mol.Wt 743. mp 152–153°C. IR (KBr) (ν_{max} cm^{-1}): 750 (P–C), 1227 (P=O), 3390 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 0.90–1.90 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12), 2.10 (s, 3H, CH_3, H-15), 3.10 (dd, 2H, CH_2, J = 4.0, 3.0 Hz, H-9), 3.40 (s, 3H, OCH_3, H-14), 3.80 (t, 2H, CH_2, J = 7.0 Hz, H-8), 4.08–4.21 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.80 (s, 1H, CH, H-4), 5.23 (dd, 1H, P–C–H, J = 2.0, 8.2 Hz, H-22), 6.60 (t, 1H, J = 7.2 Hz, NH), 7.00–8.10 (m, 7H, Ar–H), 8.30 (brs, NH, H-1); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.0 (C-4″), 15.5 (C-12), 16.5 (C-3″), 18.8 (C-15), 28.9 (C-2″), 37.2 (C-4), 39.1 (C-9), 48.3 (d, P–C–H, C-22), 54.0 (C-14), 60.2 (C-11), 64.2 (C-1″), 67.6 (C-7), 68.0 (C-8), 103.2 (C-5), 103.5 (C-3), 114.2 (C-3′), 123.4 (C-1′), 126.2 (C-5′), 127.2 (C-19), 128.1 (C-18), 131.1 (C-17), 142.3 (C-16), 142.7 (C-6), 144.2 (C-2), 166.0 (C-10), 166.1 (C-13), 126.0 (C-21), 126.4 (C-20), 131.8 (C-4′), 133.8 (C-6′), 158.2 (C-2′); ^{31}P-NMR (DMSO-d_6) δ (ppm): 21.12; ESI mass *m/z* (%) 743 (100) [$M^{+\cdot}$], 745 (66) [$M^{+\cdot}$+2], 748 (11), 725 (14), 697 (17), 610 (21). Anal. cald. for $C_{35}H_{46}Cl_2FN_2O_8P$: C: 56.53, H: 6.24, N: 3.77. Found C: 56.49, H: 6.18, N: 3.67.

*3-Ethyl-5-methyl 2-[(2-[(3-bromo-4-fluorophenyl)-(dibutoxyphosphoryl)methyl]aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate **4i***

Light yellow solid. Mol.Wt. 788. mp 198–200°C, IR (KBr) (ν_{max} cm^{-1}): 748 (P–C), 1229 (P=O), 3400 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 0.70–1.80 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12), 2.12 (s, 3H, CH_3, H-15), 3.10 (dd, 2H, CH_2, J = 4.0, 3.0 Hz, H-9), 3.50 (s, 3H, OCH_3, H-14), 3.64 (t, 2H, CH_2, J = 6.6 Hz, H-8), 4.08–4.24 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.80 (s, 1H, CH, H-4), 5.30 (dd, 1H, P–C–H, J = 1.9, 8.2 Hz, H-22), 6.10 (t, 1H, J = 7.2 Hz, NH), 7.40–8.10 (m, 7H, Ar–H), 8.50 (brs, NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm) 22.12; Anal. cald. for $C_{35}H_{46}BrClFN_2O_8P$: C: 53.34, H: 5.88, N: 3.55 Found C: 53.22, H: 5.73, N: 3.40.

*3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-[(dibutoxyphosphoryl)(4-hydroxy-3-nitrophenyl)-methyl]amino}ethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate **4j***

Orange solid. Mol.Wt: 752. mp 204–206°C. IR (KBr) (ν_{max} cm^{-1}): 747 (P–C), 1216 (P=O), 3200 (NH); ^{1}H-NMR (DMSO-d_6) δ ppm: 0.30–1.30 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12), 2.20 (s, 3H, CH_3, H-15), 3.16 (dd, 2H, CH_2, J = 4.8, 5.5 Hz, H-9), 3.40 (s, 3H, OCH_3, H-14), 3.72 (t, 2H, CH_2, J = 6.9 Hz, H-8), 4.06–4.24 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 4.60 (s, 1H, CH, H-4), 5.28 (dd, 1H, P–C–H, J = 2.0, 8.2 Hz, H-22), 6.40 (t, 1H, J = 7.8 Hz, NH), 7.20–8.10 (m, 7H, Ar–H), 8.1 (s, 1H, Ar–OH), 8.40 (brs, NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm): 23.12; Anal. cald. for $C_{35}H_{47}ClN_3O_{11}P$: C: 55.89, H: 6.30, N: 5.59. Found C: 55.80, H: 6.22, N: 5.50.

*3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-{[(dibutoxyphosphoryl)(2-thienyl)methyl]amino}ethoxy)-methyl]-6-methyl-1,4-dihydro-3,5-pyridene dicarboxylate **4k***

Black solid. Mol.Wt: 697. mp 211–213°C. IR (KBr) (ν_{max} cm^{-1}): 751 (P–C), 1231 (P=O), 3340 (NH); ^{1}H-NMR (DMSO-d_6): 0.54–1.64 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12), 2.10 (s, 3H, CH_3, H-15), 3.60 (s, 3H, OCH_3, H-14), 3.16 (dd, 2H, CH_2, J = 4.8, 5.5 Hz, H-9), 3.25 (s, 1H, CH, H-4), 3.82 (t, 2H, CH_2, J = 6.8 Hz, H-8), 4.20–4.29 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 5.30 (dd, 1H, P–C–H, J = 1.9, 8.2 Hz, H-22), 6.80 (t, 1H, J = 7.3 Hz, NH), 7.20–7.90 (m, 7H, Ar-H), 8.50 (bs, 1NH, H-1); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.6 (C-4″), 15.6 (C-12), 18.2 (C-2″), 18.4 (C-3″), 18.7 (C-15), 28.8 (C-2″), 37.2 (C-4), 39.0 (C-9), 52.9 (d, P–C–H, C-22) 54.2 (C-14), 60.2 (C-11) 61.5 (C-1″), 64.2 (C-1″), 67.9 (C-7), 68.2 (C-8), 103.1 (C-5), 103.8 (C-3), 123.2 (C-2′), 126.0 (C-3′), 126.1 (C-21), 126.4 (C-20), 126.5 (C-4′), 127.4 (C-19), 129.0 (C-18), 130.1 (C-17), 137.4 (C-1'), 141.6 (C-6), 142.3 (C-16), 144.3 (C-2), 166.4 (C-10), 166.5 (C-13); ^{31}P-NMR (DMSO-d_6) δ (ppm): 24.12.

*3-Ethyl-5-methyl 4-(2-chlorophenyl)-2-[(2-[(dibutoxyphosphoryl)(2,3-dichlorophenyl)methyl]-aminoethoxy)methyl]-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate **4l***

Green solid. Mol.Wt: 760. mp 214–216°C. IR (KBr) (ν_{max} cm^{-1}): 742 (P–C), 1232 (P=O), 3210 (NH); ^{1}H-NMR (DMSO-d_6): 0.52–1.80 (m, 17H, $4CH_2$, $3CH_3$, H-2″, H-3″, H-4″, H-12), 2.10 (s, 3H, CH_3, H-15), 3.20 (dd, 2H, CH_2, J = 4.8, 5.5 Hz, H-9), 3.60 (s, 3H, OCH_3, H-14), 3.82 (t, 2H, CH_2 , J = 7.3 Hz, H-8), 4.20–4.29 (m, 8H, $4CH_2$, H-11, H-7, H-1″), 5.10 (s, 1H, CH, H-4), 5.30 (dd, 1H, P–C–H, J = 1.9, 8.2 Hz, H-22), 6.50 (t, 1H, J = 6.0 Hz, NH), 7.20–7.80 (m, 7H, Ar–H), 8.50 (bs, 1NH, H-1); ^{31}P-NMR (DMSO-d_6) δ (ppm): 22.19; ESI mass *m/z* (%), 760 (100) [$M^{+\cdot}$], 762 (96) [$M^{+\cdot}$+2], 764 (30) [$M^{+\cdot}$+4], 766 (10) [$M^{+\cdot}$+6].

Biological assays

Antibacterial bioassay

All the synthesized compounds were screened for their antibacterial activity against gram positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* and gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* using the cup plate agar diffusion method [26]. The cultures were diluted with sterilized saline to bring the final inoculum size to approximately 10^5–10^6 CFU/mL. These solutions containing 10^6 cells/ mL were added to each Whatmann No.1 filter paper disc (5 mm diameter) and acetone and diethyl ether were used as the control. The results were compared with the activity of the standard antibiotic gatifloxacin (100 μg/mL).

Antifungal bioassay

The antifungal activity of the synthesized compounds was tested against three pathogenic fungi, namely *Fusarium oxysporium, Aspergillus niger* and *Aspergilus flavus* by the poison plate technique [27]. Test compounds were dissolved in acetone (10 mL) before mixing with potato dextrose agar (PDA, 90 mL). The final concentration of the compounds in the medium was fixed at 500 μg/ mL. Three kinds of fungi were incubated in PDA at 25 ± 1°C for 5 days to get new mycelium for antifungal assay, then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ± 1°C for 5 days. Acetone in sterilized distilled water served as control, while clotrimazole was used as positive control. For each treatment, three replicates were carried out. The radial growth of the fungal colonies was measured on the sixth day. The *in vitro* inhibiting effects of the test compounds on the fungi were calculated by the formula CV = A–B/A, where A represents the diameter of fungal growth on untreated PDA, B represents the diameter of fungi on treated PDA, and CV represents the rate of inhibition.

The bacterial and fungal cultures containing discs were placed on the media and incubated at 37°C for 24–72 h for better observation. All the experiments were carried out in triplicates and the results were expressed as zone of inhibition in mm.

Statistical analysis

Data of antimicrobial activity were expressed as means ± SD of three replicates. On the basis of the calculated value by using ANOVA method it has been observed that the difference below 0.05 level ($p < 0.05$) was considered as statistically significant.

Antiviral bioassay

Purification of tobacco mosaic virus (TMV)

Using Goodings's method [28], the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer, then filtered through double layer pledget. The filtrate was centrifuged at 10,000 × g, treated twice with PEG and centrifuged again. The whole experiment was carried out at 4°C. Absorbance values were measured at 260 nm using an ultraviolet spectrophotometer.

$$\text{Virus concn} = (A_{260} \times \text{dilution ratio})/E_{1\,\text{cm}}{}^{0.1\%,\,260\,\text{nm}}$$

Curative effect of compounds against TMV *in vivo*

Growing leaves on *Nicotiana tabacum* L. of the same ages were selected. TMV (concentration of 6×10^{-3} mg/mL) was dipped to inoculate the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then recorded 3–4 days after inoculation [29]. For each compound, three repetitions were conducted. The inhibition rate of the compound was then calculated according to the following formula ('av' means average).

$$\text{Inhibition rate}\ (\%) = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{(av local lesion numbers smeared with drugs)}}{\text{av local lesion numbers of control (not treated with compound)}} \times 100$$

The authors express their grateful thanks to UGC, New Delhi, for giving financial assistance through BSR fellowship.

The authors have declared no conflict of interest.

References

[1] B. E. Maryanoff, A. B. Reitz, *Chem.Rev.* **1989**, *89*, 863–927.

[2] R. Engel, *Chem. Rev.* **1977**, *77*, 349–367.

[3] P. Kafarski, B. Lejczack, *Curr. Med. Chem. Anticancer agents* **2001**, *1* (*3*), 301–312.

[4] S. D. Lombert, L. Blanchard, J. Jan, Y. Sakane, C. Berry, R. D. Ghai, *Biorg. Med. Chem. Lett.* **1955**, *5*, 145.

[5] J. Oleksyszyn, In: V. P. Kukhar, H. R. Hudson (Eds.), *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*, John Wiley & Sons, Chichester **2000**, 537.

[6] J. Huang, R. Chem, *Heteroatom Chem.* **2000**, *11* (*7*), 480–492.

[7] F. R. Alherton, C. H. Hassall, R. W. Lambert, *J.Med.Chem.* **1986**, *29* (*1*), 29–40.

[8] L. Jin, B. Song, G. Zhang, R. Xu, S. Zhang, X. Gao, D. Hu, S. Yang, *Bioorg. Med. Chem. Lett*, **2006**, *16* (*6*), 1537–1543.

[9] I. A. Natchev, S. Liebig, *Ang. Chem*, **1988**, *1988* (*9*), 861–867.

[10] J. Emsley, D. Hall, In: *The chemistry of Phosphorus*, Harper and Row, London **1976**, 494.

[11] (a) Hexamethyldisilazane-Al$(OTf)_3$: S. Sobhani, Z. Tashrifi, *Heteroatom Chem.* **2009**, *20* (*2*), 109–115. (b) Microreactor: E. Vam Meenen, K. Moonen, D. Acke, C. V. Stevens, *Arkivoc* **2006** *(i)*, 31–45. (c) p-TsOH: B. Kaboudin, K. Moradi, *Tetrahedron Lett.* **2005**, *46* (*17*), 2989–2991. (d) $Me2S^{+}$ $BrBr^{-}$: S. Kudrimoti, B. Vittal Rao, *Tetrahedron Lett.* **2005**, *46* (*7*), 1209–1210. (e) In$(OTf)_3$-$MgSO_4$: R. Ghosh, S. Maiti, A. Chakraborty, D. K. Maiti, *J. Mol. Catal. A.* **2004**, *210*, 53–57. (f) Ionic liquid: J. S. Yadav, B. V. S. Reddy, P. Sreedhar, *Green Chem.* **2002**, *4*, 436–438. (g) $TaCl_5$-SiO_2: S. Chandrasekhar, S. J. Prakash,

V. Jagedeshwar, C. Narasimhulu, *Tetrahedron Lett.* **2001**, *42* (*32*), 5561–5563.

[12] S. Laschat, H. Kunz, *Synthesis* **1992**, *23* (*8*), 90–95.

[13] (a) J. Zon, J. Pol, *J. Chem.* **1981**, *55*, 643. (b) B. C. Ranu, A. Hajra, J. Jana. *Org. Lett.* 1999, *1* (*8*), 1141–1143.

[14] E. Ruiz, H. Rodriguez, J. Coro, E. Salfran, M. Suarez, R. Martnez-Alvarez, N. Martin, *Ultrason. Sonochem.* **2011**, *18*, 32–36.

[15] J. Safaei-Ghomi, M. Fadaeian, A. Hatami, *Turk. J. Chem.* **2007**, *31*, 89–95.

[16] G. A. Heropoulos, S. Georgakopoulos, B. R. Steele, *Tetrahedron Lett.* **2005**, *46* (*14*), 2469–2473.

[17] J. L. Luche, *Synthetic Organic Sonochemistry*, Pienum Press, New York, USA **1999**, Ch. 2, pp. 55–56.

[18] H. A. Stefani, R. Cella, F. A. Dorr, C. M. P. Depereia, F. P. Gomes, G. Zeni, *Tetrahedron Lett.* **2005**, *46*, 2001–2003.

[19] L. C. Thomas, *The Interpretation of the Infrared Spectra of Organic Phosphorus Compounds*, Heyden and Sons, London **1974**, 129.

[20] P. Bennker, U. S. Pat. Appl. 09/809, 351, Oct 9, (**2001**).

[21] K. Reddimohan Naidu, K. R. K. Kumar Reddy, Ch. Vinod Kumar, D. Divya Sharma, C. Naga Raju, *S. Afr. J. Chem.* **2009**, *62*, 185–188.

[22] G. C. S. Reddy, B. S. Kumar, A. U. R. Sankar, M. V. N. Reddy, C. S. Reddy, *Korean Chem. Soc.* **2008**, *52* (*6*), 657–662.

[23] D. Petersen, M. Marcolini, L. Bernadi, F. Fini, P. R. Herrera, V. Sgarzani, A. Ricci, *J. Org. Chem.* **2006**, *71*, 6269–6272.

[24] B. Sadek, K. M. S. Fahelelbom, *Molecules* **2011**, *16*, 4339–4347.

[25] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th ed., Butterworth, Heinemann, Oxford **1997**, OX2, 8DP.

[26] A. L. Barry, *The Antimicrobial Susceptibility Test: Principle and Practices*, Edited by Illus lea and Febiger, Philadelphia, PA, USA **1976**, p. 180; [Biol. Abstr. 1977, 64, 2518 3].

[27] S. Q. Song, L. G. Zhou, D. Li, D. Tang, J. Q. Li, W. B. Jiang, *Nat. Prod. Res. Dev.* **2004**, *16*, 157–159.

[28] G. V. Gooding, T. T. Hebert, *Phytopathology* **1967**, *57*, 1285–1290.

[29] S. Z. Li, D. M. Wang, S. M. Jiao, **1991**, 93–94.

